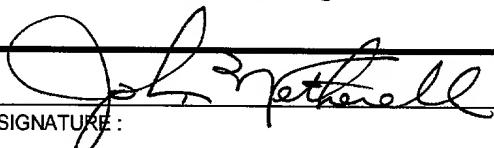


SUBSTITUTE FORM PTO-1390  <b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE  ATTORNEY'S DOCKET NUMBER 12724-002001	
		U.S. APPLICATION NO. (If Known, see 37 CFR 1.5) <b>09/719889</b>	
INTERNATIONAL APPLICATION NO. PCT/AU99/00495	INTERNATIONAL FILING DATE 18 June 1999	PRIORITY DATE CLAIMED 18 June 1998	
TITLE OF INVENTION <b>METHOD OF TISSUE REPAIR II</b>			
APPLICANT(S) FOR DO/EO/US Earl R. Owen, Peter Maitz, Rodney I. Trickett, Judith M. Dawes, James A. Piper, Peter Dekker			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> <li><input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input checked="" type="checkbox"/> This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).</li> <li><input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).</li> <li><input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))             <ol style="list-style-type: none"> <li><input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li> <li><input type="checkbox"/> has been communicated by the International Bureau.</li> <li><input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li><input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</li> <li><input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))             <ol style="list-style-type: none"> <li><input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li> <li><input type="checkbox"/> have been communicated by the International Bureau.</li> <li><input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li><input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li><input type="checkbox"/> An English language translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li><input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li><input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol>			
Items 11 to 16 below concern other documents or information included:			
<ol style="list-style-type: none"> <li><input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li><input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li><input type="checkbox"/> A FIRST preliminary amendment.</li> <li><input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</li> <li><input type="checkbox"/> A substitute specification.</li> <li><input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li><input checked="" type="checkbox"/> Other items or information:             <ul style="list-style-type: none"> <li><input checked="" type="checkbox"/> The claims submitted have been amended under Article 34</li> <li><input type="checkbox"/></li> <li><input type="checkbox"/></li> <li><input type="checkbox"/></li> <li><input type="checkbox"/></li> </ul> </li> </ol>			
<div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;"> <b>CERTIFICATE OF MAILING BY EXPRESS MAIL</b> </div> <div style="display: flex; justify-content: space-between;"> <span>Express Mail Label No. <u>EL688321817US</u></span> <span></span> </div> <div style="font-size: small; margin-top: 5px;">         I hereby certify under 37 CFR §1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231       </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <span><u>December 18, 2000</u></span> <span><u>Derek Norwood</u></span> <span>Derek Norwood Typed Name of Person Signing</span> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <span>Date of Deposit</span> <span>Signature</span> </div>			

U.S. APPLICATION NO. (IF KNOWN) <b>09/719889</b>	INTERNATIONAL APPLICATION NO. PCT/AU99/00495	ATTORNEY'S DOCKET NUMBER 12724-002001		
17. <input type="checkbox"/> The following fees are submitted:		<b>CALCULATIONS PTO USE ONLY</b>		
<b>Basic National Fee ( 37 CFR 1.492(a)(1)- (5) ):</b>				
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... <b>\$1000</b>				
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$860</b>				
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$710</b>				
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... <b>\$690</b>				
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... <b>\$100</b>				
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>		<b>\$1,000.00</b>		
Surcharge of <b>\$130</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		<b>\$0.00</b>		
Claims	Number Filed	Number Extra	Rate	
Total Claims	- 20 =		x <b>\$18</b>	<b>\$0.00</b>
Independent Claims	- 3 =		x <b>\$80</b>	<b>\$0.00</b>
<b>MULTIPLE DEPENDENT CLAIMS(S) (if applicable)</b>			+ <b>\$270</b>	<b>\$0.00</b>
<b>TOTAL OF ABOVE CALCULATIONS =</b>		<b>\$0.00</b>		
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.		<b>\$500.00</b>		
<b>SUBTOTAL =</b>		<b>\$500.00</b>		
Processing fee of <b>\$130</b> for furnishing the English Translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f))		<b>\$0.00</b>		
<b>TOTAL NATIONAL FEE =</b>		<b>\$500.00</b>		
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +		<b>\$0.00</b>		
<b>TOTAL FEES ENCLOSED =</b>		<b>\$500.00</b>		
		<b>Amount to be refunded:</b>	\$	
		<b>Charged:</b>	\$	
a. <input checked="" type="checkbox"/> A check in the amount of \$500.00 to cover the above fees is enclosed.				
b. <input type="checkbox"/> Please charge my Deposit Account No. 06-1050 in the amount of \$0.00 to cover the above fees. A duplicate copy of this sheet is enclosed.				
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.				
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.</b>				
SEND ALL CORRESPONDENCE TO:				
 John R. Wetherell, Jr., Ph.D. FISH & RICHARDSON P.C. 4350 La Jolla Village Drive, Suite 500 San Diego, CA 92122 (858) 678-5070 phone (858) 678-5099 facsimile				
SIGNATURE: _____ NAME: _____ John R. Wetherell, Jr., Ph.D. REGISTRATION NUMBER: _____ 31,678				

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JC01 Rec'd PCT/PTO 18 DEC 2000

APPLICATION  
FOR  
UNITED STATES LETTERS PATENT

TITLE: METHOD OF TISSUE REPAIR II

APPLICANT: EARL R. OWEN, PETER MAITZ, RODNEY I. TRICKETT,  
JUDITH M. DAWES, JAMES A. PIPER, PETER DEKKER

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December 18, 2000

Date of Deposit

Signature

Derek Norwood

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WO 99/65536

- 1 -

**METHOD OF TISSUE REPAIR II****Technical Field**

5 The present invention relates to methods for joining living tubular tissues; organs and their coverings; skin and appendages; as well as the various internal and peripheral nerves of the body, the spinal cord and its ramifications. The invention also relates to a solder for use in those methods and methods for preparing the solder.

10

**Background Art**

In repairing living tissues, sutures or clips are routinely used to close defects, join planes of tissues or to join bodily tubes together (anastomoses).

15

This involves the placing of materials in the body which cause some damage to the tissues involved, but hold those tissues in apposition while the body's own healing processes effect a more permanent join. The damage that various joining materials cause varies but even careful placement of microsutures in the smallest of bodily tubes during an anastomosis produces a fibrous tissue reaction around each of the suture materials left in situ.

20

Joins, however made, take time, and those joins made by placing individual sutures in tubular joins are the most time consuming. Sewing in a ring of sutures to effect such a join inside the body may demand a large incision to obtain the access required to effect enough surgical freedom to manipulate the equipment and instruments required. Microsuturing requires considerable skill.

30

**Arteries and Other Tubes**

Fluids, and materials suspended within them, can travel along the body's patent tubes. Arteries carry blood from the heart to other organs and tissues in the body. They have 3 layers, an inner specialised mucosa (termed the

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+61 2 62832634

WO 99/65536

PCT/AU99/00495

- 2 -

intima), a thicker, middle, muscular and structural layer which contains collagen and elastin connective proteins (the media), and an outside layer which is a scaffold with fibrous tissue, blood vessels and nerves all supplying the 5 functions of the artery (the adventitia). The inner volume of the artery is the lumen.

For tubes such as arteries to function in transporting blood at high pressure, they need to be strong. They are actually active in transporting a pressure wave of blood by 10 expanding and relaxing (systole and diastole) as the bolus of blood passes. Joining such active tubes requires such physiological activity as promoting blood flow to be considered and the design of methods of anastomosis that will allow the activity to continue after the join.

15 Injuries to an artery are potentially very serious for an animal or human, as blood flowing through the artery is at high pressure and blood loss can be rapid. If the intima layer is damaged, then the middle, structural layer, the media, is exposed to blood. This triggers an important 20 repair mechanism which acts to seal the wound and prevent further bleeding by the formation of blood clots on the wound, caused by blood coming into contact with the exposed collagen of the media.

Although microsuturing is the standard clinical repair 25 technique for a severed artery, it has several disadvantages. A high skill level is required to make between 6 and 12 separate sutures to repair the artery. The sutures remain in the body acting as a site for fibrous tissue to form due to foreign body reaction, and this 30 fibrous tissue is a point of weakness in the artery even after it is deemed to have healed. Although suturing does not produce a fluid-tight seal, surgeons usually rely on blood clotting triggered by the mechanism described above to seal the vessel soon after the repair is complete.

35 A number of laser-assisted welding techniques have been explored in order to find a more convenient technique

+61 2 62832634

WO 99/65536

PCT/AU99/00495

- 3 -

which does not lead to so much scarring. These almost always need stay sutures (sutures used to join the vessels before laser treatment, which may or may not be removed subsequently) for a successful outcome. In this case the 5 two vessel ends are held together to allow stay sutures to be inserted and then a laser is used to heat the tissue at the join so that proteins at the site are coagulated and bond together. Lasers such as the infra-red holmium-doped 10 YAG and carbon dioxide lasers have been used because these produce wavelengths which are strongly absorbed by water in the tissue. Alternatively a dye solution may be applied to the tissue to enhance light absorption at a suitable laser wavelength. In any case, it is crucial that the intima 15 layers of the 2 ends are in continuity, to avoid a blockage or a clot and to promote smooth laminar flow in the repaired vessel. This is difficult to achieve in thick-walled vessels where the laser energy may not be absorbed through all three layers of the vessel to form a strong 20 weld with a smooth intima layer.

25 Some protein glues have been used to repair blood vessels, such as fibrin (which triggers a blood clotting reaction to effect a tissue join). A possible disadvantage of such a glue is the potential to be associated with blood clotting within the vessel, partially or wholly obstructing it.

30 Laser-activated fluid albumin solder has also been used, but the solder has required stay sutures to achieve sufficient repair strength for arteries which carry blood at high pressure. Fluid glues and solders tend to run between the tissue ends, risking blockage of the inner lumen, and are difficult to control and position accurately 35 on the tissue repair. To attain a seal, they have been applied circumferentially around the join, which is then circumferentially welded. These joins later show thick scarring which can cause stricture or blockage of the vessel or tube.

+61 2 62832634

WO 99/65536

PCT/AU99/00495

- 4 -

There is also a lack of precision in such techniques, because of differences in the glue or fluid solder consistency, variations in the type of applicator device used to apply the glue or fluid solder, and the pressure 5 needed to form a join.

A major drawback with current fluid solders is that they rapidly deteriorate and change composition when introduced into moist environments.

Similarly, existing solid solders must be kept dry 10 when introduced to moist arteries, to prevent them from absorbing moisture, weakening their internal bonding and losing strength, even though this occurs more slowly than for fluid solders.

The repair of other bodily tubes is similar in 15 concept. Since the structure of each tube is specialised to its function and the nature of its contents, there must be careful choice of the method of tube repair so that it will not interfere with the tube function, and in particular with maintaining the inner lumen of the tube.

20

#### Peripheral Nerves

The electrical signals that control the body's organs and transmit information back and forth to the central nervous system (CNS) travel along peripheral nerves.

25

A peripheral nerve has an outer membrane consisting of connective tissue such as collagen. This membrane (epineurium) protects and holds separate bundles of nerves or fascicles together. The fascicles group together nerve axons supplying a specific region of the body and are 30 bounded by perineurium membranes. Each axon is supported by a Schwann cell within the fascicle. Nerve metabolism is sustained by the vascular system from both outside and within the nerve..

35

When a peripheral nerve is cut all axons distal (further from the spine) to the wound change their properties. Even when the nerve is reconnected, these axons

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WO 99/65536

- 5 -

PCT/AU99/00495

continue to degenerate distally. The Schwann cells which normally wrap themselves around the axons as insulation, guide regenerating axons. Joining nerves as accurately as possible by lining up corresponding fascicles enables the 5 enclosed axons to more efficiently regenerate.

Peripheral nerves can have diameters ranging from approximately 1cm to approximately 50 micrometres.

10 Operating on nerves and other tissues of small dimensions has been facilitated by using magnification and special microsurgical equipment. Accurate nerve repairs need to be effected at the fascicular level ensuring that regeneration is along the correct bundle leading to the 15 original area those axons supplied.

The current technique of peripheral nerve repair uses 15 microsuturing. This technique requires a dedicated, trained surgeon as microsuturing of just one of the many fascicles with three or more microsutures (using say a 70 micron diameter needle and 30 micron thread) can take very 20 long operating times. There is the prospect of added damage to the inner axons due to sutures penetrating the thin perineurial sheath. The use of sutures results in some 25 scarring of the repair due to foreign body reaction. Excessive scarring impairs nerve function and may be associated with painful neuromas. There is also evidence, that in the long term, scar tissue formation and scar 30 maturation can impair the joined nerve.

Work has been performed on the use of lasers alone in 35 effecting nerve joins. To date the welds have typically been made using infrared lasers such as carbon dioxide lasers which rely on water absorption for energy transfer. Tissue preparation before welding relies on overlapping the nerve membranes. One of the problems of laser welding has been the fact that the intact axonal tissue is under pressure within the fascicle, so that when it is cut the 35 axons extrude. Laser treatment can thus lead to

+61 2 62832634

WO 99/05536

PCT/AU99/00495

- 6 -

denaturation of the axon material leading to scarring and proliferation of fibrous tissue.

Laser-activated protein solders have also been tried, as described for the artery and blood vessel case above.

5 Again because of difficulties in controlling fluid solders, and the weakness of the resulting bonds in a moist environment, these repairs are usually too weak without the addition of stay sutures. This complicates the surgical technique and leads to additional scarring and foreign body reaction.

10 The bonds formed to date as described in the prior art using laser welding have typically lacked strength and thus microsuturing has been used in addition to welding to strengthen these joins.

15 Solutions to at least some of these problems are taught in WO96/22054. The present invention relates to alternative solutions.

#### DESCRIPTION OF THE INVENTION

20 In a first aspect the present invention provides a biomolecular solder comprising an at least substantially solid composition of at least one biomolecule which has been mixed at high concentration with an aqueous solvent, which composition is treated to at least partially denature 25 the biomolecular component(s) of the solder and to at least partly dry the solder.

The biomolecule(s) is typically proteinaceous but it is envisaged that other naturally occurring biomolecules could be used as alternatives. Further, analogues of 30 biological, biodegradable polypeptides could be used. Analogues of biological, biodegradable polypeptides useful in the solders of the invention include synthetic polypeptides and other molecules capable of forming the solder of the invention but which do not cause adverse 35 reaction in the tissue undergoing repair.

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WO 99/65536

PCT/AU99/00495

- 7 -

Where the biomolecule is a protein, the protein can be any protein or mixture of proteins but is preferably biodegradable in the relevant host. Examples of suitable proteins include albumins, collagen, fibrinogen and 5 elastin. Suitable proteins are typically those which can be cross-linked to form a matrix and which can be resorbed by the body. Where combinations of proteins are used it is envisaged that those combinations will be of proteins having similar denaturation temperatures. An example is the 10 combination of albumin and collagen. Use of different albumins is contemplated including bovine, horse, human, rat, ovine and rabbit albumin. The choice of a particular albumin may be made to reduce immunological reaction in the patient to the solder. It is envisaged that there will be 15 circumstances where the albumin used may be chosen to match the patient's blood type and possibly even more specifically with regard to histocompatibility markers of the patient in question.

The solvent is typically water but other aqueous 20 solvents including saline may be used provided that any salt etc present does not adversely affect the solder upon denaturation.

The solder can be formed from a protein paste made up of highly concentrated protein in an aqueous solvent which 25 is typically water. Highly concentrated protein encompasses protein concentrations in the range of 40 to 80% w/w. Preferably the protein concentration is in the range of 45 to 75% w/w. More preferably, the protein concentration is in the range of 50 to 60% w/w. The range 30 of 50 to 60% is especially preferred for bovine serum albumin, or rat or rabbit or ovine or human albumin. The starting concentration of protein loses water (or aqueous solvent) as it dries or is dried during processing. The prepared solder may contain little or no solvent.

35 It is preferred to incorporate light-absorbing material, such as a dye, into the solder, to improve

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WO 99/65536

- 8 -

PCT/AU99/00495

energy deposition in the solder. An example of a suitable dye is indocyanine green which is preferably incorporated at a concentration within the range 0.1 to 2.5% w/w. Other suitable dyes include methylene blue and fluorescein isothiocyanate. It will be understood that the light-absorbing material is chosen to be appropriate to the energy source that is used in forming tissue repairs involving the use of the solder. The light absorbing substance may be incorporated by being added to the solvent and dissolved in it prior to addition of the biomolecule(s) to the solvent.

In one embodiment the solder is prepared from a composition of:

15 55-75% w/w albumin  
45-25% w/w water  
0.25% w/w indocyanine green

The albumin may be bovine, rabbit, human, ovine or rat albumin.

20 The at least partial denaturation of the biomolecule(s) substantially reduces the solubility of the solder. Typically the biomolecule(s) of the solder is denatured to a sufficient extent to ensure that the solder will have sufficient longevity *in vivo* for the repair, for which the solder is being used, to be formed. Denaturation favourably alters the mechanical properties of the solder so that on moistening it exhibits similar mechanical properties to the tissue under repair. The denaturation can be effected by heat, light, radiation, ultrasound or chemical means. Typically the heat denaturation is 25 carried out in an aqueous environment such as in a water bath in steam or in pressurised steam. Without wishing to be bound by theory, the present inventors believe that the aqueous environment permits at least partial denaturation without dissolution and with the maintenance of 30 "structural" water involved in the integrity of the 35

+61 2 62832634

WO 99/65536

PCT/AU99/00495

- 9 -

biomolecule(s). Denaturation may be effected before, during or after shaping of the solder.

The solder can be provided in a variety of shapes. In particular, the solder of the invention is suitable for extruding into tubular forms, a form that cannot readily be achieved with prior art solders. It can also be extruded into a partial tube which has a curved cross section with an elongate open channel which can be wide or narrow. The solder can be prepared with a smooth surface or with a surface that is at least slightly roughened. Roughening may be of assistance in enhancing contact between tissue and solder. The roughening may provide a profile which appears smooth at macroscopic level but rough at microscopic level. The tubular and partially tubular forms typically have a round or ovoid profile but other profiles are also contemplated including square, crenulated and other geometric forms. The tubular solder of the invention can be tapered or of uniform cross section. The tubular solder of the invention is well suited to nerve repair applications and is particularly well suited to vascular applications in which the moisture content makes prior art solders unsuitable. The solder can be prepared in other shapes as required for particular applications including strips, patches, solid rods and hollow tubes with at least one flanged end.

Various adjuvants can be added to the solder to promote rapid or more complete tissue healing, eg fibrinogen (for blood vessels), growth factors, sodium hyaluronate (for improved viscous handling and possibly better healing), hormones, and/or anticoagulants, such as heparin.

Various fibrous materials can be added to the solder to improve the strength of the solder [eg collagen or polytetrafluoroethylene fibre (which is sold under the brand names goretex and teflon) or ceramic fibres]. The fibres are typically biocompatible polymers. The

denaturation of the solder with fibrous materials within it may be by chemical means (such as with acid or hydroxide) or by heat and could include bonding of the protein to the fibres.

5 The solder need not be of uniform composition throughout. In some applications it will be desirable to include one or more adjuvants in one or more parts of the solder and not in others. Similarly, it may be desirable to incorporate fibres in some parts and not others or else 10 different fibres in different parts. Further, one or more light-absorbing substances may be incorporated in some parts of the solder and not others or the light-absorbing substance may be incorporated at different concentrations throughout one or more parts of the solder. It will be 15 recognised that such variations may be particularly useful with various shaped forms of the solder such as tubes. Still further, different parts of the solder may be denatured to different extents and different parts of the solder may be provided with different surface textures, 20 such as being smooth in some parts and at least slightly roughened in other parts.

The solder can be applied to a mesh, stiffener or graft material made from, for instance, a metal, synthetic fibre or plastic. Because of its pliability, the solder 25 may be embedded into spaces in the mesh or it may be applied as a covering to all or part of the mesh, stiffener or graft material. In one embodiment, it may be applied only to the ends of a graft material, mesh or stiffener to effect welding of the graft material, mesh or 30 stiffener to the appropriate tissue.

The formation of such materials may involve coextrusion or coating of a biologically inert porous structure (such as a goretex tube or shape) with solder. Where a coating is utilised in this embodiment, the solder 35 may be initially formulated in a fluid form, that is, with a substantially lower concentration of the biomolecule(s).

+61 2 62832634

WO 99/65536

- 11 -

PCT/AU99/00495

The fluid solution is applied, allowed to dry and may be reapplied and allowed to dry before being at least partially denatured. The drying process reduces the solvent content so that the final consistency of the 5 solder is the same as that achieved by forming the solder from the high concentration solution as described above.

The solder of the invention can be introduced to the relevant tissue by the surgeon, and placed in the correct position, using forceps. If necessary, the solder can be 10 cut to a required size or shape during surgery.

The at least partially denatured biomolecule(s) of the solder has strong internal bonding and is substantially unaffected by water absorption. Any water absorption that occurs acts to enhance the flexibility of 15 the solder rather than causing its dissolution or disruption.

The solder can be introduced into the relevant tissue in an appropriately moistened form. In this form the solder is flexible and will not fracture when cut, 20 squeezed or manipulated with surgical instruments.

The solder can be sterilised after denaturing and before use, by for instance gamma ray irradiation, for instance at 2000 rad/min for 50 minutes. Other suitable forms of sterilisation include autoclaving, steam 25 treatment and heat treatment.

Activation of tissue bonding by the solder is induced by heat. This can be achieved in a variety of ways but laser activation is the most common. Because the biomolecule(s) is already at least partially denatured, 30 dissolution is at least substantially prevented, allowing time for more complex manipulations to be completed. Laser activation of bonding through overlying tissue is possible with this solder, that is, the solder can be applied under, over, or under and over the tissue to be 35 joined.

+61 2 62832634

WO 99/65536

PCT/AU99/00495

- 12 -

In a second aspect the present invention provides kits of solder tubes, partial tubes and shapes formed from solder of the first aspect of the invention. The kits may comprise tubes, partial tubes and/or shapes of different sizes to suit different surgical applications. The different sized tubes can include different lumen sizes, wall thicknesses and lengths. It is envisaged that tubes will often be cut to length to suit the repair to be effected during surgery thus minimising the number of different lengths that need to be provided. The kits can include tubes, partial tubes or shapes fashioned from solders made with different biomolecules, including those made with biomolecules which reflect the need to match the repair material for histocompatibility markers in the animal or human patient in which the repair is to be made. Further the tubes, partial tubes or shapes can be provided in different versions including a series of different adjuvants, light-absorbing substances and/or fibres as well as with different solder compositions throughout the tubes, partial tubes or shapes.

In a third aspect the present invention provides a method of preparing a solder of the first aspect, the method comprising the steps of forming a high concentration solution of one or more biomolecule(s) in an aqueous solvent, at least partially denaturing the biomolecule(s) and drying the solder.

Typically the method includes forming the solid solder into a shape which is preferably a hollow tube. Other suitable shapes include partial tubes, strips, patches, hollow tubes with at least one flanged end or solid rods suitable for the tissue being repaired.

To form hollow tubes, the solder can be extruded into hollow tubes by the use of a high pressure extrusion and die set, manufactured of stainless steel or other suitable biologically inert material, which may have very smooth surfaces to permit smooth solder shapes to be extruded.

+61 2 62832634

WO 99/65536

- 13 -

PCT/AU99/00495

Shaped solders can also be prepared by injection moulding. Alternatively, the extruded solder may be prepared with an at least slightly roughened surface to enhance contact between the solder and the tissue to which it is applied.

5 In this form, the solder may have a surface which is roughened on a microscopic scale but appears smooth on a macroscopic scale. The tube dimensions can be in the range of 0.2mm to 6cm in diameter, with variable wall thickness, which depending on the tube diameter and strength of the 10 solder, can be as low as 50  $\mu$ m. It will be understood that for veterinary applications, where very large animals and very small animals may be involved that even greater diversity of tube sizes may be required to suit the needs of various physiological tubes in need of repair. The 15 solder of the invention is suited to the precision manufacture of tubes of desired dimensions.

In one embodiment, the method for forming a tubular solder comprises forming a high concentration solution of at least one biomolecule in an aqueous solvent, extruding 20 the solution without permitting it to dry, allowing the extruded material to dry, at least partially denaturing the extruded material, allowing the at least partially denatured, extruded material to dry, moistening the material, cutting the material to length, finally drying 25 the material and sterilising the material.

The starting concentration of biomolecule loses aqueous solvent as it dries or is dried during processing. In the prepared solder, little or no solvent may be present.

30 The method may include incorporating a light-absorbing material, such as a dye, into the solder, to improve light energy deposition in the solder, with the light-absorbing material being chosen to be appropriate to the energy source that is used in forming tissue repairs 35 involving the use of the solder. Where a light-absorbing material is incorporated this may be achieved by mixing

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WO 99/65536

- 14 -

PCT/AU99/00495

the light absorbing substance into the solvent and then adding this solution to the biomolecule(s) for mixing.

Indocyanine green dye (for example, prepared at a concentration of 0.25mg/ml where the solder is placed over the tissue to be joined and 2.5mg/ml where the solder is placed under the tissue to be joined) can be incorporated into albumin protein paste (approximate concentration on mixing 60% weight/weight) which is then preferably denatured by the immersion of the protein solder in a water bath at elevated temperature (preferably around 85°C) for a suitable period of time (preferably 30 seconds) or in steam where temperatures over 100°C are used.

Typically the biomolecule(s) of the solder is denatured to a sufficient extent to ensure that the solder will have sufficient longevity *in vivo* for the repair for which the solder is being used to be formed. The denaturation can be effected by physical means such as heat (direct or indirect), light, radiation or ultrasound or chemical means. Typically the denaturation is carried out in an aqueous environment such as a water bath in steam or in pressurised steam. This can be achieved where the biomolecule(s) is proteinaceous by immersing the protein paste in hot liquid (preferably water) at a temperature of over 40°C [preferably 85°C for bovine serum albumin (BSA)] for a suitable time (preferably 30 seconds for BSA) or in steam where temperatures over 100°C are used, for a suitable period of time. For human or rabbit serum albumin, steam treatment by for instance autoclaving at temperatures between 100°C and 150°C are preferred, with temperatures between 110°C and 130°C being more preferred. An example of a suitable temperature is about 120°C. The steam treatment is typically for about 10 minutes. Denaturation may be effected before, during or after shaping of the solder.

The method can include the addition of various adjuvants to the solder, eg fibrinogen (for blood

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WO 99/65536

- 15 -

PCT/AU99/00495

vessels), growth factors, sodium hyaluronate (for improved viscous handling and better healing), hormones, and/or anticoagulants, such as heparin.

The method can also include the incorporation of 5 various fibrous materials into the solder to improve the strength of the solder (eg collagen or polytetrafluoroethylene fibre, or ceramic fibres). The fibres are typically biocompatible polymers. The denaturation of the solder with fibrous materials within 10 it may be by chemical means (such as with acid or hydroxide) or by heat and could include bonding of the biomolecule(s) to the fibres.

The method may be modified to produce a solder that is not of uniform composition throughout. For instance, 15 in some applications it will be desirable to include one or more adjuvants in one or more parts of the solder and not in others. Similarly, it may be desirable to incorporate fibres in some parts and not others or else different fibres in different parts. Further, one or more 20 light-absorbing substances may be incorporated in some parts of the solder and not others or the light-absorbing substance may be incorporated at different concentrations throughout one or more parts of the solder. A gradient or profile of the concentration of light-absorbing material 25 can be provided within the solder to control the heat deposition within the solder and avoid excessive thermal tissue damage. The gradient can be created during the preparation of the solder or by painting on a dye solution after solder tube formation. Still further, different parts 30 of the solder may be denatured to different extents. Still further, the solder may be prepared with part of the surface at least partly roughened and part of the surface smooth.

The method may include sterilising the solder after 35 denaturing and before use. Suitable means of sterilisation include gamma ray irradiation, for instance

+61 2 62832634

WO 99/65536

- 16 -

PCT/AU99/00495

at 2000 rad/min for 50 minutes, autoclaving, steam treatment, heat treatment and gas sterilisation.

Final denaturation of the solder occurs *in situ* in the tissue, by application of laser or other energy source,

5 where the energy is absorbed by the solder and/or the tissue.

In a fourth aspect the present invention provides a method of repairing a biological tissue comprising the use of a solder of the first aspect in effecting the repair.

10 The method can be used for effecting repairs in animal as well as human patients.

Typically, the method involves the use of an energy source such as a laser for effecting tissue joins using the solder. Where the energy source is a laser, the selected 15 laser has a wavelength appropriate to any light-absorbing substance used to concentrate the energy at the repair site. The laser chosen should also be appropriate to the tissue being repaired in that the tissue absorbs the energy produced by the laser poorly. For blood vessels, the 20 combination of diode lasers with indocyanine green dye is appropriate. The energy provided should be sufficient to bond the solder to the underlying or overlying tissue while minimising damage to the underlying tissue. The power used will vary for different tissues and can be matched to the 25 amount of energy output required to effect bonding.

The time of treatment for each bond to be effected can vary depending on such factors as ambient conditions, altitude, humidity and the nature of the tissue being joined as well as the moisture level of the tissue being 30 joined.

In one embodiment the invention provides a method for joining body tubes combining the use of a tubular solder of the first aspect and a laser fusion device. The tubular solder can be applied (depending on the physiological tube 35 to be repaired) to either fit inside or outside or inside and outside both the cut ends of the tube. The lasering

WO 99/65536

PCT/AU99/00495

- 17 -

may be done either directly or through the living tube to the solder to change its characteristics to make it adhesive.

5 The solder tube can incorporate a light-absorbing material to absorb the wavelength of the laser beam which is applied to form the bond.

Bonding can involve attaching at least one edge of the circumference of the solder tube to the inside or outside of the cylindrical surface of a body tube. The 10 join of the body tube can be completed by placing both ends of the tube within the solder tube and applying energy through the solder to bond the solder to the underlying tissue or by placing both ends of the body tube over the solder tube (Figure 10) and applying energy 15 through the overlying tissue to the solder or by placing one end of the body tube within the solder tube and one end over the solder tube and applying energy to effect bonding. Where the tube to be repaired includes a damaged section which requires replacement a graft material with 20 solder applied at least at the ends can be joined at either end to a free end of the severed tube (Figure 9).

Where the tissue repair is with respect to nerve tissue or other tissue tubes where the tube contents need to be protected from damage, it is especially important 25 that the weld should not be concentrated on the edges being joined as this can damage extruded tissue. Rather, the weld should be transverse to the edge of the discontinuity.

The solders of the invention can be used, in conjunction with suitable promoters of neuron growth, in 30 tubular form, to provide guides for nerve regeneration. In this use the severed nerve ends are inserted into the ends of the tube and welded in place.

The solders of the invention can also be used in 35 tubular form with a sealed end as a cap for the ends of severed nerves to assist patients who experience

discomfort, which can be extreme, where severed nerves cannot be rejoined, for instance, in amputation stumps.

Where the tissue to be repaired is an essentially wide hollow body tube, the repair can comprise the

5 insertion of a thin-walled hollow cylinder of bio-degradeable solder inside the tube under repair so that the cylinder spans the severed portions of the tube.

End-to-end repairs can also be performed by pulling one end of the repair site through the tube and folding

10 back a cuff of tissue over the tube and then sleeving the other end over the cuff and effecting welds to hold the tube and ends in place. It will be understood that in this particular method it is necessary for the energy source chosen to effect the weld to propagate through the overlying tissue.

Repairs of tubes in accordance with the invention can include end-to-side as well as end-to-end tubular repairs.

End to side repairs can be performed by providing a tube with a flange at one end adapted to fit into a x-

20 shaped incision in the side of the tube into which the end is to be inserted. The free tubular end of the solder tube is attached to the end of the tube to be inserted into the x-shaped incision. The sides of the x-shaped incision are welded around the circumference of the solder tube to seal 25 the insertion site. The end-to-side join can be at a variety of angles and thus the flanged portion of the tube can be provided at the appropriate angle for the join to be formed.

The repair methods of the invention may be utilised 30 for joining a diversity of living tubular tissues including arteries, veins, lymphatics, microvessels, any of the body's tubes such as its ducts - pancreatic, liver, cystic, tear, prostatic, and the ureters, urethra, epididymis, vas, fallopian tubes, bowel, bronchi and other 35 gastroenterological and respiratory and body and brain ducts and tubes.

+61 2 62832634

WO 99/65536

PCT/AU99/00495

- 19 -

The repair method of the invention can also be applied to the repair of organs and their coverings such as liver, spleen, kidney, uterus, testicles, bladder, cystic, 5 correal, brain and other capsules, coverings and skin and appendages, as well as the various internal and peripheral nerves of the body, the spinal cord and its ramifications by use of at least one appropriately shaped solder of the invention for the repair being made.

10

The present invention provides a new system of laser-solder-fusion, with or without control of the laser operation which we have demonstrated to be suitable for joining together to produce usual function, in severed 15 living tubes in the rodent, namely arteries, veins, nerves and the vas deferens and bowel. Not only are these severed tubular structures joined without subsequent leakage, but they function immediately after joining, those joins are at least eventually as strong and long lasting as is possible 20 with appropriate sutures, they are able to be joined in an exceptionally short time and in addition this is done without inflicting the trauma occasioned by other methods. The system can be adapted to be used through equipment now 25 and in the future developed for minimally invasive therapies.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a solid protein cylinder of the invention measuring 2mm in length and 1.1 mm inner diameter and 1.3 30 mm outer diameter.

FIGURE 2 shows a schema of an operative technique of the fourth aspect of the invention: (A) The solder is pushed over the proximal vessel end and the vessel wall is pulled back. (B) Laser energy application at the distal part of 35 the solder. (C) The distal end of the vessel is gently pulled over the entire length of the solder. (D) Laser

energy application to the proximal part of the solder.

FIGURE 3 shows the appearance of the laser welded micro-anastomosis immediately after clamp release (A) and after 6 weeks (B).

5 FIGURE 4 shows graphic representation of tensile strength of suture and laser solder anastomoses of rat aortas as a function of time after surgery. (time is on logarithmic scale)

10 FIGURE 5 shows a laser-welded anastomosis in longitudinal section immediately after laser irradiation. (Masson's trichrome, (A) arrow indicates direction of blood flow, 5x magnification and (B) 50x magnification)

15 FIGURE 6 shows the remains of solder in the vessel wall after 6 weeks. Note the normal appearance of the intima and media. Note the presence of phagocytotic cells at the solder surface. (Toluidine Blue, magnification 20 x)

FIGURE 7 shows scanning electron micrographs of the lumen of the laser-welded anastomosis 10 minutes after reestablishing perfusion (longitudinal section).

20 (magnification x 100).

FIGURE 8 is a schematic cross section of an anastomosis of a blood vessel formed using the sleeve technique.

FIGURE 9 shows a graft in side and cross sectional view formed using the sleeve technique of the fourth aspect of 25 the invention at both ends of the graft.

FIGURE 10 shows in schematic form, a join formed by placing a solder tube of the invention inside a body tube. Solder strips may be used externally to strengthen the anastomosis.

30

#### BEST METHOD OF CARRYING OUT THE INVENTION

WO 99/65536

PCT/AU99/00495

- 21 -

## 1. PREPARATION OF SOLDER

## Starting Composition:

protein	55-75% (w/w)
water	45-25% (w/w)
dye	0.25% (w/w)

5

The protein is bovine, rabbit, human, ovine or rat albumin. Suitable concentrations for bovine serum albumin include about 55% and for human and rabbit albumin include about 57%. Indocyanine green is a suitable dye. Albumins can be obtained from Sigma-Aldrich Corporation. Suitable albumin preparations include:

Bovine albumin - A 2153 Fraction V powder (minimum 96%);  
Human albumin - A 1653 Fraction V powder (96-99% albumin);  
Rabbit albumin - A 0639 Fraction V powder;  
15 Sheep albumin - A 3264 Fraction V powder;  
Horse albumin - A 9888 Fraction V powder.  
- ovine albumin.

Indocyanine green dye can be obtained from Becton Dickinson Microbiology Systems, Maryland 21030 USA.

20

A particular formulation for human and rabbit albumin is as follows:

## Starting Composition:

25	albumin	57.3% (w/w)
	water	42.45% (w/w)
	ICG dye	0.25% (w/w)

## Construction:

1. the components (accurately measured) are mixed into a paste form to obtain optimum consistency for extrusion or pressing. For example, the water and dye are first mixed by vortexing to form a consistent dye solution which is then added to the protein followed by mixing to form the paste. Mixing can be performed physically or mechanically and for small batches (<2g total mass) was performed using a vortex mixer to

WO 99/65536

PCT/AU99/00495

- 22 -

provide consistency. The solder was not allowed to dry at this stage as this would cause the solder to become brittle and thus unsuitable for extrusion or pressing.

2. The paste can be extruded at this stage but as 5 noted below a superior product can be achieved by deferring final shaping.

3. The extruded paste was then allowed to dehydrate thus increasing the protein concentration and allowing the solder to take a more rigid form.

10 4. The rigid solder was immersed in hot water at 80-90°C (for example 85°C for bovine albumin) for approximately 1 minute to denature the protein. Where the solder is prepared from human or rabbit albumin the relevant treatment is with steam at about 120°C for 10 15 minutes (it is envisaged that the temperature could be as low as 100°C or up to 150°C). This denaturation treatment causes the solder to bond within itself and the solder becomes less soluble in water.

5. The solder at this stage is elastic and may be 20 further cut into desired shapes easily without inducing stress or fracture. Desired shapes include sheets, tubes, partial tubes and rods. If cut to shape before step 4, the solder may fracture through the presence of crystalline structure if it is too dry or else it may deform if it is 25 too moist.

6. The solder is preferably dehydrated at this stage and gamma irradiated or autoclaved for sterilisation and stored in a dry, sterile and light proof container.

30 A particular protocol that has been used successfully with the human or rabbit serum albumin formulation mentioned above is:

1. mix the protein preparation
2. extrude the preparation
- 35 3. allow the preparation to dry

WO 99/65536

PCT/AU99/00495

- 23 -

4. autoclave the preparation at 120°C for 10 minutes.

## 2. METHOD OF REPAIR

The following repairs have been effected:

5

Rat aorta: 1.3 mm diameter

cylinder used: 1.4 mm internal diameter  
1.7 mm external  
diameter. 2 mm length

Rabbit femoral

artery: 2mm diameter

Cylinder used: 1.6 mm internal diameter  
2.1 mm external diameter  
2mm length

Joining tubes can involve attaching at least one edge of the circumference of a solder tube to the inside or outside of the cylindrical surface of a body tube. The 10 join of the body tube can be completed by placing both ends of the tube within the solder tube and applying energy through the solder to bond the solder to the underlying tissue or by placing both ends of the body tube over the solder tube and applying energy through the 15 overlying tissue to the solder or by placing one end of the body tube within the solder tube and one end over the solder tube and applying energy to effect bonding. Where the body tube to be repaired includes a damaged section which requires replacement a graft material with solder 20 applied at least at the ends can be joined at either end to a free end of the severed tube.

WO 99/65536

PCT/AU99/00495

- 24 -

Where the tissue repair is with respect to nerve tissue or other tissue tubes where the tube contents need to be protected from damage, it is especially important that the weld should not be concentrated on the edges being joined as this can damage extruded tissue. Rather, the weld should be transverse to the edge of the discontinuity.

End to side repairs can be performed by providing a tube with a flange at one end adapted to fit into a x-shaped incision in the side of the tube into which the end is to be inserted. The free tubular end of the solder tube is attached to the end of the tube to be inserted into the x-shaped incision. The sides of the x-shaped incision are welded around the circumference of the solder tube to seal the insertion site. The end-to-side join can be at a variety of angles and thus the flanged portion of the tube can be provided at the appropriate angle for the join to be formed.

End-to-side repairs can also be performed by providing a partial solder tube with a flange adapted to fit over a longitudinal incision in the side of the body tube onto which the new tubular end is to be attached. The sides of the longitudinal incision are pulled through the solder flange, everted around the flange and welded to the outside of the solder flange. The free end of the side branch is then pulled over the previously welded body tube and flange and welded to the main body of the partial solder tube. The main body of the partial solder tube is then welded to the outside of the main body tube. The end-to-side join can be at a number of angles and thus the flanged portion of the tube can be provided at the appropriate angle.

Repairs of non-tubular tissues are effected by using at least one appropriately shaped solder of the invention together with an energy source to effect bonding between solder and tissue.

WO 99/65536

- 25 -

PCT/AU99/00495

## 3. DESCRIPTION OF SLEEVE METHOD

The proximal artery (tube) is pulled through a tube of solder and turned back on itself a short distance using purpose built forceps, which have ends adapted to provide a 5 surface which functions to maintain the tube end in open form, such as the forceps illustrated in Figure 2. The overlapping turned back artery is lasered to an observable slight change in colour and specific temperature, which denatures the protein and causes it to adhere to the vessel 10 wall on both or at least one side in a circle around the proposed join area. The distal artery (or tube) is slightly stretched and manipulated gently over the already lasered area and beyond to the as yet unlasered solder tube of 15 equal lasing area. This area is then lasered in the same way and causes that circular portion of the artery to be lasered to the cylinder. That completes the join.

## Example 1

A total of 90 rats were divided into two groups 20 randomly. In group one the anastomoses were performed using conventional microsuturing technique, while in group two the anastomoses were performed using our new laser welding technique. In addition, each of the two groups were divided into 5 subgroups and evaluated at different followup 25 periods (10 min, 1 hour, 1 day, 1 week and 6 weeks). At these intervals the anastomoses were evaluated for patency and strength (Tensile strength measurement). 3 anastomoses in each subgroup were processed for light and electron microscopy.

30 All anastomoses were found to be patent. The mean clamp time of the anastomoses performed with conventional suturing was 20.6 minutes compared to 7.2 minutes for the laser activated welded anastomoses ( $p<0.001$ ). The strain measurements showed a stronger mechanical bond of the 35 sutured anastomoses in the initial phase. However, at 6 weeks the tensile strength of the laser welded anastomoses

WO 99/65536

PCT/AU99/00495

- 26 -

was higher compared to the conventional suture technique. Histologic evaluations revealed a near complete resorption of the solder after six weeks. The junction site of the vessel ends could not be determined on the luminal side of 5 the artery.

In conclusion, a resorbable protein used as a solder, activated by a diode laser, can provide a reliable, safe and rapid arterial anastomosis, which could be performed by any microsurgeon faster than conventional suturing after a 10 short learning curve.

Simplifying vascular anastomoses in surgery and in particular in small diameter vessels has been an important topic in the past. A recent publication reviewed the technical developments in this field since the start of 15 this century [1]. Minimising foreign body reaction at the anastomotic site has been an important issue, and a variety of authors have described the negative impact of suture materials, staples and clips on vessel wall compliance and active force production [2-6]. The use of laser welding 20 techniques for vascular anastomosis has first been reported by Jain in 1979 [7,8]. Different types of lasers have been used [9-12] in order to minimise the potential negative impact on tissues. Most reported techniques require at least three permanent stay sutures and therefore laser 25 welding was used only to seal the vessel and not to mechanically hold the vessel ends together. The use of lasers to weld tissue relies on the efficient deposition of heat due to the light absorbed by the tissue. The laser wavelengths that have been used thus correspond to strong 30 absorption bands of water, hemoglobin or other tissue chromatophores. The introduction of dyes such as indocyanine green [13,14] or fluorescein isothiocyanate [15] enhances the delivery of the laser energy precisely to the target tissues. In addition, the application of laser 35 activated protein solders has been shown to strengthen laser welds in tissues such as nerves [16-18]. Our study

WO 99/65536

PCT/AU99/00495

- 27 -

5 presents a sutureless, quick and reliable technique to successfully anastomose small diameter arteries, avoiding vessel wall fibrosis by eliminating any permanent implanted devices. We combined an anastomotic technique reported by Payr in 1900 [19] with the use of a fully biodegradeable, diode-laser-activated protein tube to weld small diameter arteries.

#### MATERIALS AND METHODS

10 A total of 90 young adult male Wistar rats (outbred) weighing 450 to 550 g were used in this study. Consent and approval for this investigation were obtained from our Institution's Animal Ethical Review Committee. All surgical procedures were performed under general anaesthesia with a  
15 halothane/oxygen mix (4% halothane at 4 L/min oxygen for inducing and 2% halothane at 2 L/min oxygen for maintaining anaesthesia). Clean, but not aseptic conditions were maintained during the surgical procedures, which were performed using a Zeiss OPMI 7 operating microscope. A  
20 midline laparotomy was performed and the infrarenal aorta exposed, incising the peritoneum, freeing the tissues and ligating lumbar and ileolumbar vessels if necessary. A double microvascular clamp (Edward Weck Inc., micro vessel approximator 1.5 mm x 8.0 mm blades, 19 mm bar) was applied  
25 to the aorta, which was severed with straight microscissors. After flushing the two stumps with saline, connective tissue in excess was removed, but leaving the adventitia intact. In 45 animals the anastomoses were carried out by conventional microsuturing (9/0 Nylon with a  
30 140  $\mu$  needle, 10 to 12 interrupted sutures) and in the remaining 45 animals the anastomoses were performed by laser welding. The clamp time of all procedures was recorded for later statistical analysis with the students t-test. No local or systemic anticoagulant drugs were used,  
35 nor were the animals given antibiotics post-operatively.

## LASER WELDING

A GaAlAs laser diode with a nominal power of 250 mW and wavelength of 805 nm (Spectra Diode Labs Inc., San Jose CA) was used. The laser radiation was coupled into a 100  $\mu\text{m}$  diameter core, numerical aperture (NA 0.28) optical fiber, which was held by hand in a fiber chuck. The diode current and temperature were controlled by a SDL-800 diode driver. The diode was operated by a foot switch and was set at 90 mW during surgery, with a spot size at the tissue of 200  $\mu\text{m}$  diameter, corresponding to a maximum irradiance of 286  $\text{W/cm}^2$  at the tissue surface. The laser power was measured with a Scientech power meter (Boulder Inc., CO USA). The total irradiation time for each circular weld was 10 sec approximately.

The solder used in this study was a mixture of water, concentrated bovine serum albumin and indocyanine green (ICG) dye (Becton Dickinson, Maryland USA). ICG has a maximum absorption coefficient at a wavelength of 805 nm of  $2 \times 10^5 \text{M}^{-1}\text{cm}^{-1}$ . ICG binds preferentially with serum proteins such as albumin [20] ensuring that the heat is efficiently transferred to denature the protein solder. A high protein concentration mixture (55.40% albumin: 44.33% water: 0.27% ICG by weight starting material) was obtained by vigorous stirring of the components. The mixture was formed into tubes suited to the dimensions of a rat aorta. The solder tubes were predenatured to make them more flexible and chemically stable (Figure 1).

The solid protein tube was then used in a way similar to that described by Payr in 1900 when using absorbable magnesium rings [19], (Figure 2). The proximal vessel was passed through the cylinder, everted over the edge for a length of 1 mm and then welded to the protein cylinder by means of laser energy, further denaturating the protein contained in the solder (Figure 2 A, B). Laser energy was delivered by an optical hand-held fiber for a period of

WO 99/55536

PCT/AU99/00495

- 29 -

time according to the tissue reaction visible through the operating microscope (approximately 10 sec/ circumference). When the slightest retraction of the tissue was noted the laser spot was moved to adjacent tissue until the total circumference of the vessel was welded onto the protein cylinder. The two branches of the double clamp were then approximated and the distal vessel was gently pulled over the entire protein cylinder (Figure 2 C). Laser energy was then applied to create a bond between the distal end of the artery and the most proximal part of the solder (Figure 2 D).

Immediately after removing the clamps the anastomoses were examined to assess patency by the milking test. Each group was then divided into 5 subgroups to be reevaluated at different intervals (10 minutes, 1 hour, 1 day, 1 week, 6 weeks) with 9 animals per subgroup. At the chosen time all anastomoses were re-exposed and patency was checked with the milking test. In 6 animals per subgroup the anastomotic sites together with 5 mm of vessel proximally and distally were removed and subjected to tensile strength measurements. These were performed by attaching one end of the vessel to a calibrated force transducer (FT30C, Grass Instruments, Quincy, MA) and the other end to a screw driven translator [18]. In 3 animals per subgroup the vessels were clamped, flushed with saline and fixative (5% glutaraldehyde buffered to pH7.4) and finally removed for histology. Staining for light microscopy was done with Masson's trichrome to clearly differentiate native protein from denatured protein and with Toluidine Blue. Scanning electron microscopy was used to study the inner surfaces of the anastomoses.

#### RESULTS

All animals survived the surgical procedure and all anastomoses were patent at the time of re-exploration. At 6 weeks there were no aneurysms at the site of the sutured or

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WO 99/65536

PCT/AU99/00495

- 30 -

laser welded anastomoses (Figure 3).

The mean clamp time of the sutured anastomoses was 20.6 minutes (SD 2.82, SEM 0.52) which was significantly longer than the mean clamp time of the laser welded anastomoses, 7.2 minutes, (SD 2.26, SEM 0.41), ( $p < 0.001$ ; students t-test).

Tensile strength measurements revealed that the sutured anastomoses were stronger (under stress) when compared to the laser-welded anastomoses in the short term (134.6 gm and 45.3 gm respectively). However, at 6 weeks the tensile strength for the laser welded anastomoses was slightly higher in comparison to the sutured anastomoses (134.2 gm and 103.9 gm respectively,  $p = 0.005$ , student's  $t$ -test) (Figure 4) The sutured anastomoses, when subjected to traction, ruptured at the junction level tearing a small cuff off the vessel wall, while the laser-welded vessels detached at the distal portion of the bond, probably the weakest point of the anastomosis.

Light microscopy evaluation after staining of the anastomotic site immediately after laser application with Masson's trichrome revealed denatured protein in the layer directly adjacent to the solder, but no changes could be observed in the media of the artery (Figure 5). After 6 weeks the solder was almost completely resorbed and the intimal layer could be observed in continuity. Healing occurred with proliferation of myofibroblasts and the site of the anastomosis could not be detected from the lumen of the artery (Figure 6). However, some fibrotic reaction could be seen on the adventitia as shown in Figure 3 b, but again the media of the artery did not reveal any changes. Scanning electron microscopy of the anastomotic site after perfusion was reestablished for 10 minutes showed some red blood cell deposition at the site of the anastomosis, but this did not have any impact on patency (Figure 7).

## DISCUSSION

Since Jain's first report on the successful use of 5 laser energy for the repair of blood vessels [7], there have been numerous attempts to develop a technique for sutureless anastomosis of blood vessels employing laser welding. The advantages of laser welding have been shown to be due to a perfect seal of the junction with no leakage 10 and less foreign body reaction due to less suture material. However, the need for stay sutures to maintain vessel end approximation has led to the term laser-assisted anastomosis [21-25], where the laser is used to seal an 15 anastomosis after three to five stay sutures have been previously inserted. On the other hand, true sutureless anastomoses have been performed by using an intraluminal stent to ensure intimal alignment [26-28]. These stents have mostly been designed to be intraluminally absorbed and therefore may potentially lead to arterial embolism and/or 20 thrombosis. A previously reported technique to repair tubular structures [18] employs protein solder bands containing indocyanine green dye, which were designed to absorb the laser energy and therefore heat was localized at the protein solder and the immediate surrounding tissue. 25 Changes in the tissue due to heating caused by the laser energy were observed only in the tissue layer in immediate contact with the solder. In order to get optimal intimal alignment, which is crucial for successful microvascular anastomosis, the protein solder was extruded 30 into a tube with corresponding diameters to the vessel to be repaired. The optimal incimal alignment was accomplished by employing a technique introduced by Payr at the turn of the century [19]. This technique was further developed by Landon [29] eliminating the need for ligatures to secure 35 the vessel onto the ring and by Carter [30] for coronary artery surgery using a polyethylene ring. Haller [31]

WO 99/65536

PCT/AU99/00495

- 32 -

reported a 92 % patency rate in the anastomosis of 4-mm diameter vessels using Payr's technique with tantalum rings. This technique prevents the blood coming in contact with the protein solder which eliminates the risk that the 5 coagulation cascade is activated and leads to smooth intimal alignment. The laser welding technique causes the tissue to bond to the protein solder tube by means of protein denaturation in the tissue and the solder.

In earlier studies the actual mechanism of the bond 10 created by laser welding has been identified as the possible homogenisation of the adventitia as well as coagulation necrosis of smooth muscle cells, however the elastic lamellae were unaltered [32]. In a direct laser welding study, protein denaturation of the collagen fibers 15 was observed with electron microscopy, with a slight interruption of the intima and subsequent re-endothelialization within 10 days [33]. Dehydration of the triple helix molecular structure of collagen present in the arterial wall breaking Van der Waal's bonds, which subsequently re-form to other collagen molecules, was 20 reported as a possible bonding mechanism [34]. Our technique confines the laser-induced changes in the artery wall to the layer directly in contact with the protein solder, thus minimizing any weakening of the vessel wall. 25 In particular neither the proximal nor distal vessels' tunica media were altered by the laser energy as shown by histologic evaluation. It may then be suggested, that by this technique the arterial wall is only minimally altered and does not lose its mechanical properties. After healing 30 of the anastomotic site the tensile strength measurements revealed better results for the laser-welded anastomoses compared to the sutured anastomoses, which could be a result of the fibrous reaction to the suture material in the tunica media.

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WO 99/65536

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CLAIMS:

1. A substantially solid biomolecular solder comprising biomolecules which are denatured so that in use, the solubility of the solder is reduced.

5 2. A solder according to claim 1 wherein the biomolecule is a protein.

3. A solder according to claim 2 wherein the protein is any one or more of albumin, elastin, collagen and fibrinogen.

10 4. A solder according to any one of the preceding claims, further comprising a dye for improving energy deposition into the solder when the solder is exposed to energy.

5 5. A solder according to claim 4 wherein the dye is indocyanine green, methylene blue or fluorescent isothiocyanate.

6. A solder according to any one of the preceding claims, further comprising an adjuvant for promoting rapid or more complete tissue healing.

20 7. A solder according to claim 6 wherein the adjuvant is a growth factor, sodium hyaluronate, a hormone or an anti-coagulant.

8. A solder according to any one of the preceding claims, further comprising a material for improving the 25 strength of the solder.

9. A solder according to claim 8 wherein the material is a polytetrafluoroethylene fibre or a ceramic fibre.

10. A kit comprising a solder according to any one 30 of the preceding claims.

11. A method of preparing a biomolecular solder, the method comprising the following steps:

(a) forming a substantially solid composition comprising biomolecules and a solvent;

35 (b) denaturing the biomolecules in the composition; and

(c) drying the composition to form the solder; wherein in step (b), the biomolecules are denatured so that in use, the solubility of the solder is reduced.

12. A method according to claim 11 wherein in step 5 (b) the biomolecules are denatured by exposing the composition to energy for a time period which is sufficient to allow the energy to denature the biomolecules.

13. A method according to claim 12 wherein the 10 energy is thermal energy.

14. A method according to claim 13 wherein the biomolecules are denatured by heating the composition at a temperature of greater than 40°C for a time period of about 30 seconds or longer.

15. A method according to claim 14 wherein the composition is heated in a hot liquid bath or in pressurised steam.

16. A method according to claim 11 wherein in step (b), the biomolecules are denatured by exposing the 20 composition to a compound for a time period which is sufficient to allow the compound to denature the biomolecules.

17. A method according to claim 11 wherein in step (a), the substantially solid composition is formed by 25 mixing the biomolecules with a solvent in amounts which are sufficient to allow the substantially solid composition to form.

18. A method according to claim 17 wherein the biomolecules and the solvent are mixed in amounts of 30 80%w/w and 20%w/w respectively.

19. A method according to claim 11 wherein in step (a), a dye for improving energy deposition into the solder is added to the substantially solid composition.

20. A method according to claim 19 wherein the dye 35 is added to the composition in an amount between 0.1 to 2.5% w/w.

- 39 -

21. A method according to claim 20 wherein the dye is mixed with the solvent, prior to mixing the solvent with the biomolecules.

5 22. A method according to claim 11 wherein in step (c), drying the composition to form the solder removes all of the solvent from the solder.

10 23. A method according to claim 11 wherein the composition is formed into a shape before the biomolecules in the composition are denatured in step (b).

24. A method according to claim 23 wherein the composition is applied to a structure before the biomolecules in the composition are denatured in step (b).

15 25. A method according to claim 24 wherein the structure is a mesh, stiffener or graft material.

26. A method according to claim 11 further comprising the step of sterilizing the solder.

27. A method of repairing a biological tissue, the method comprising the following steps:

20 (a) applying a solder according to claim 1 to the site of a tissue to be repaired; and

(b) exposing the solder to energy for a time sufficient to allow the solder to bond to the tissue so that the tissue is repaired.

25 28. A method according to claim 27 wherein the solder is moistened before application to the site of the tissue to be repaired.

## ABSTRACT

A substantially solid biomolecular solder for joining tissue comprising a partially denatured biomolecule (e.g. protein or polypeptide). The solder can be formed into shapes, e.g. tubes, to suit. The invention also relates to methods for joining tissue and methods for preparing the solder.

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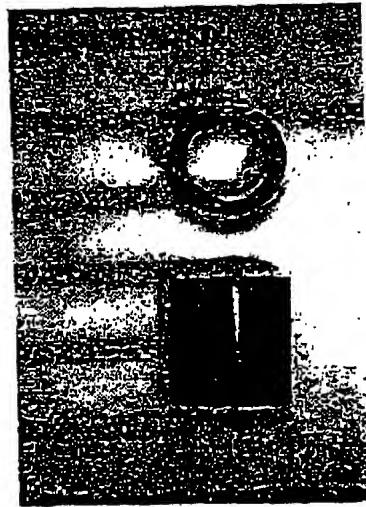


FIGURE 1

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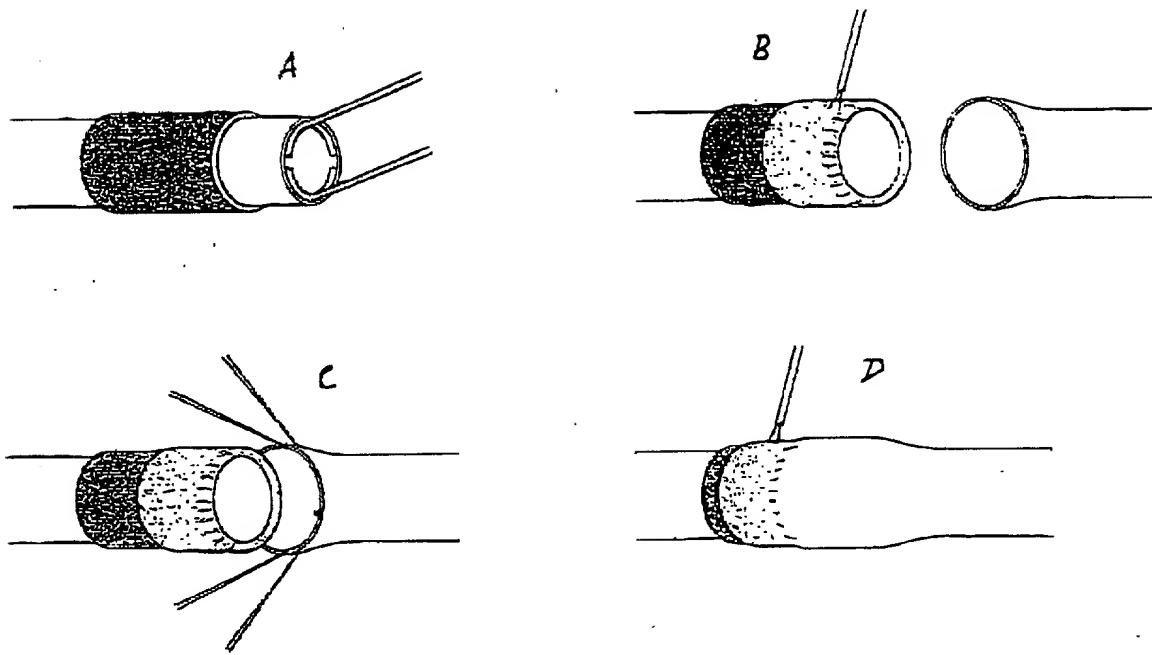


FIGURE 2

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↓ Blood flow

FIGURE 3A

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Blood flow

FIGURE 3B

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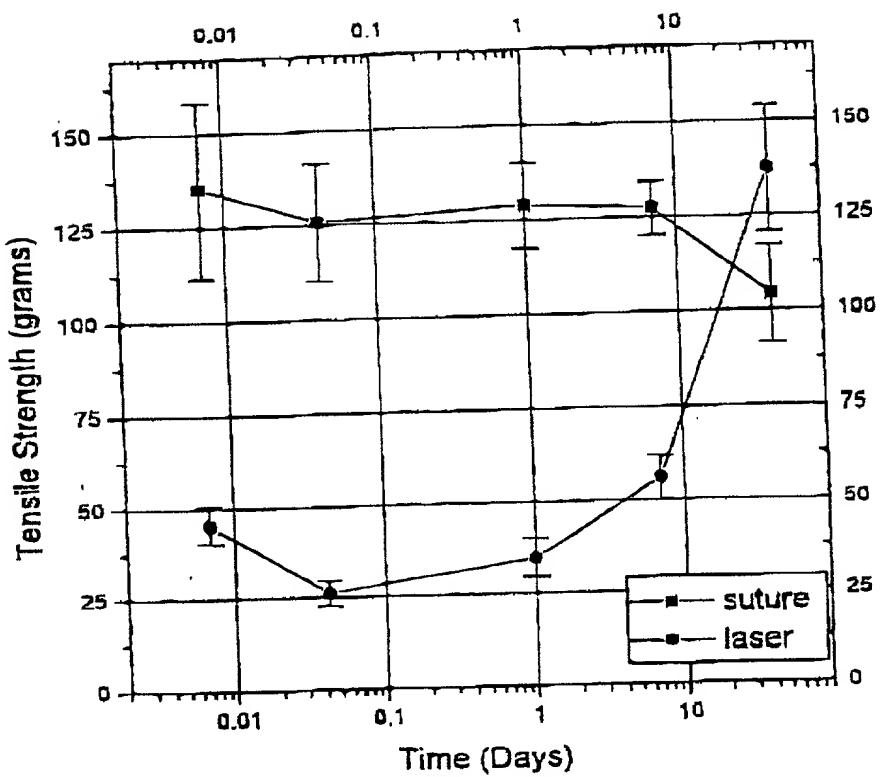


FIGURE 4

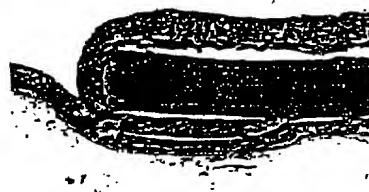
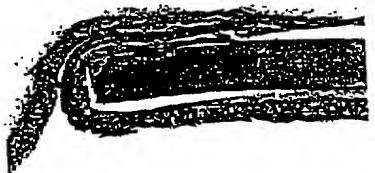
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Blood flow

FIGURE 5A

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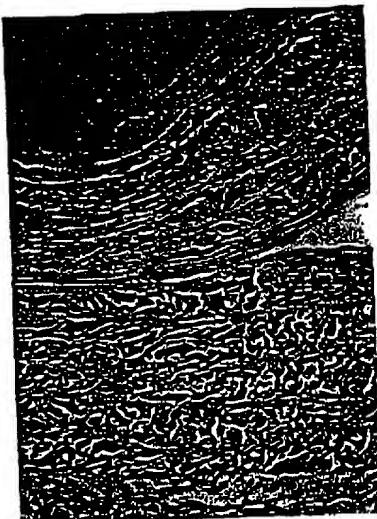


FIGURE 5B

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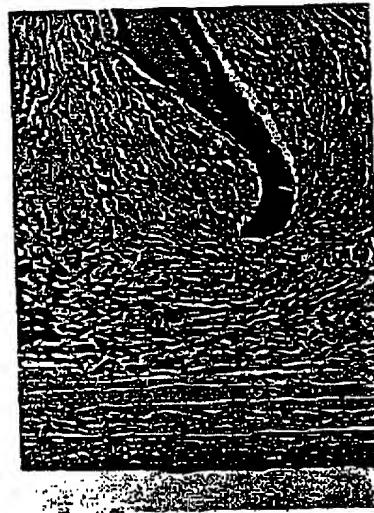


FIGURE 6

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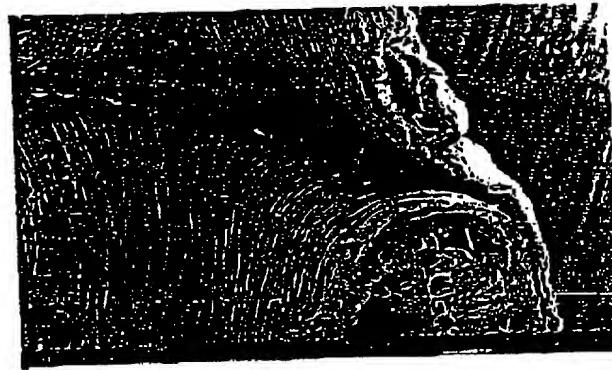


FIGURE 7

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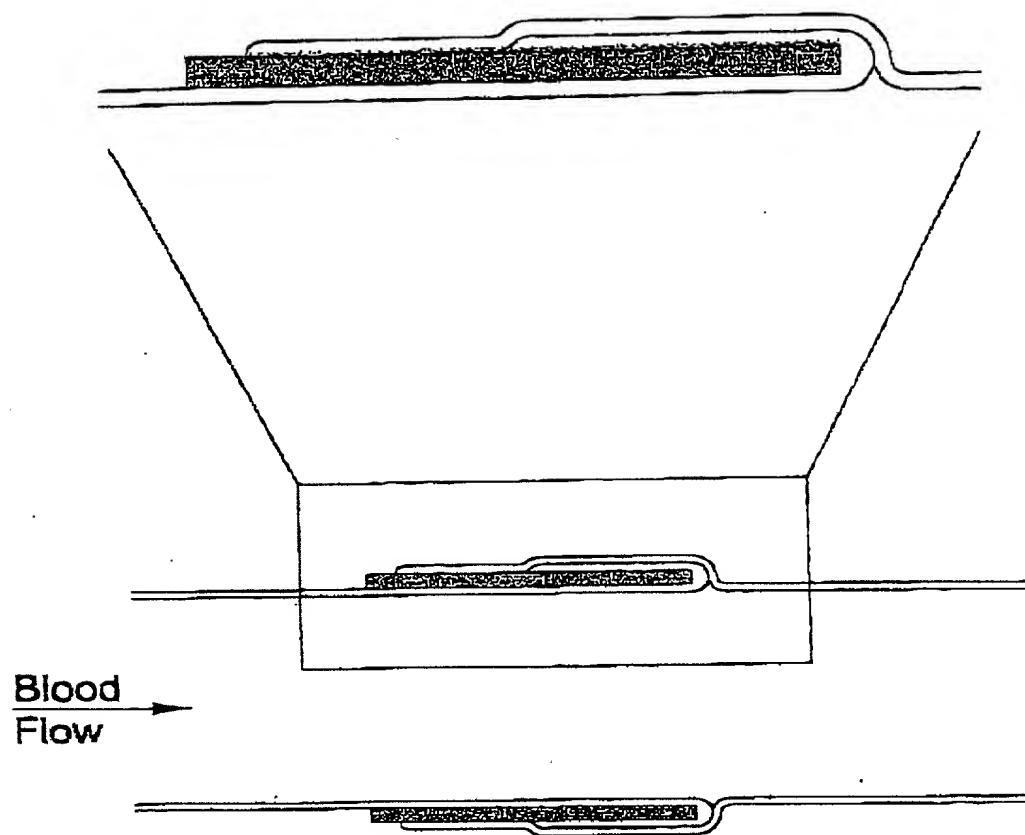


FIGURE 8

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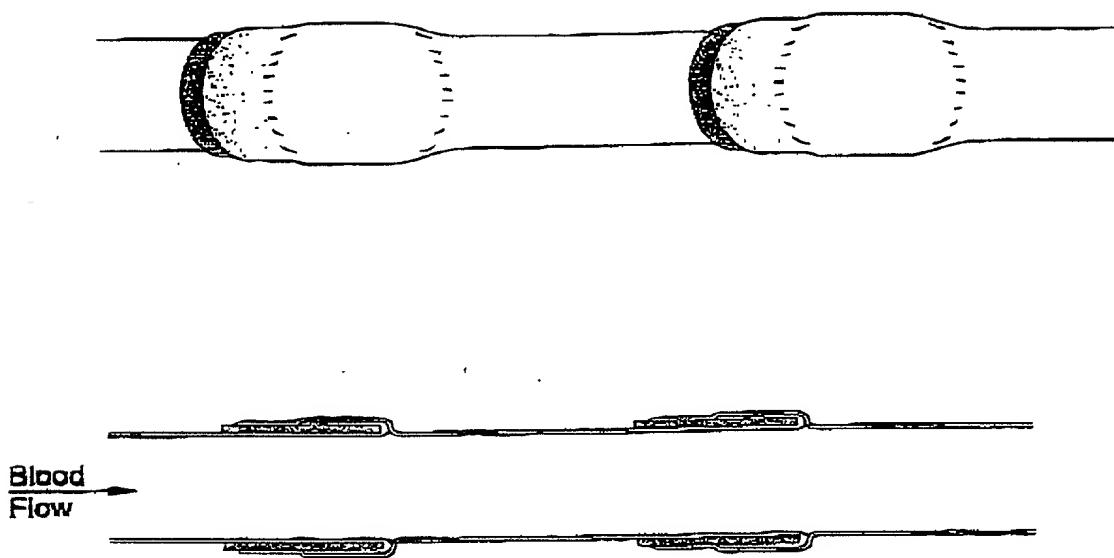


FIGURE 9

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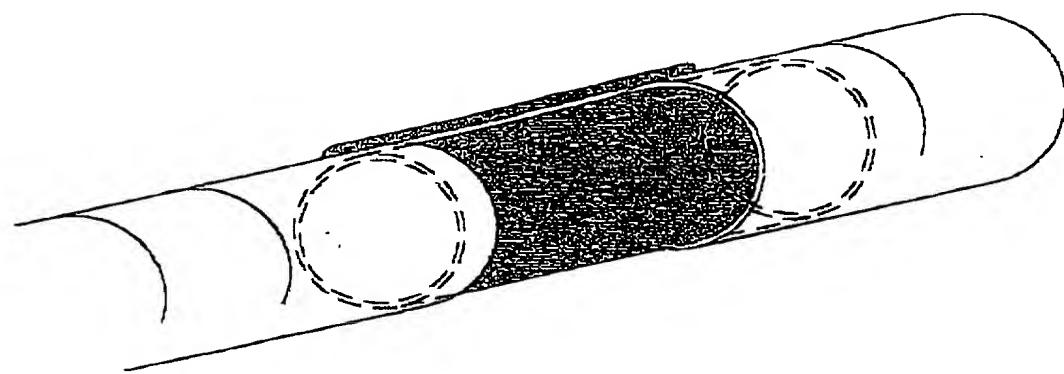


FIGURE 10

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As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD OF TISSUE REPAIR II, the specification of which:

is attached hereto.

was filed on December 18, 2000 as Application Serial No. 09/719,889 and was amended on \_\_\_\_\_.

was described and claimed in PCT International Application No. \_\_\_\_\_ filed on \_\_\_\_\_ and as amended under PCT Article 19 on \_\_\_\_\_.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

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U.S. Serial No.	Filing Date	Status
None		

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Country	Application No.	Filing Date	Priority Claimed
PCT	PCT/AU99/00495	June 18, 1999	<input type="checkbox"/> Yes <input type="checkbox"/> No
Australia	PP 4214	June 18, 1998	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

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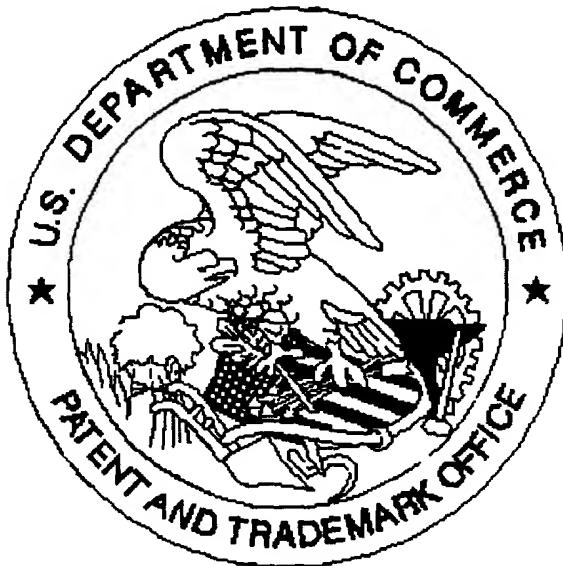
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